ACMG Classification Rules Specified for Fibrillin-1 (FBN1) Variants

Gene	Disease (MONDO ID)	Transcript
FBN1	MONDO: 0007947	NM_000138

Summary of ACMG-AMP Criteria for Fibrillin-1 (FBN1) Variants

PATHOGENIC CRITERIA		
Criteria	**Criteria Description**	**Specification**
VERY STRONG CRITERIA		

PVS1	Null variant in a gene where loss of function is a known mechanism of disease.
	• Follow the adapted flowchart
	• There is only 1 relevant transcript for FBN1 (NM_000138)
	• The C-terminal region is proven to be critical to protein function (multiple LP/P variants identified in this region)
	• Add caveat that PP3 cannot be applied if using the PVS1 criterion for splice site variants in position +/- ½
	• In practice:
	  ○ Nonsense/frameshift variants predicted to undergo NMD (not affecting last exon or 55 last nt of penultimate exon)
	  ○ 1,2 splice site variants leading to exon skipping or use of a cryptic splice site disrupting the reading frame and predicted to undergo NMD
	  ○ Full gene deletion
	  ○ Single to multi-exon deletion disrupting the reading frame and predicted to undergo NMD
	  ○ Duplication (>=1 exon in size and completely contained within gene) proven in tandem and disrupting the reading frame and predicted to undergo NMD
		Disease-specific

PS2_very strong PM6_very strong	*De novo* (paternity and maternity confirmed) in a patient with the disease and no family history • Use the SVI WG point-based system https://clinicalgenome.org/working-groups/sequence-variant-interpretation/	Strength

	• For further definition see PS2	
STRONG CRITERIA		
PVS1_strong	Null variant in a gene where loss of function is a known mechanism of disease. • Follow the flowcharts, per SVI working group recommendation • In practice: ○ Nonsense/frameshift variants predicted to escape NMD (affecting last exon, last 55nt of the penultimate exon) ○ 1,2 splice site variants leading to exon skipping or use of a cryptic splice site disrupting the reading frame and predicted to escape NMD ○ 1,2 splice site variants leading to exon skipping or use of a cryptic splice site but preserving the reading frame ○ Single to multi-exon deletion disrupting the reading frame and predicted to escape NMD ○ Single to multi-exon deletion preserving the reading frame ○ Duplication (>=1 exon in size and completely contained within gene) presumed in tandem and presumably disrupting the reading frame and predicted to escape NMD	Strength
PS1	Same amino acid change as a previously established pathogenic variant regardless of nucleotide change. • Add caveat: beware of changes that impact splicing rather than the amino acid. Splicing predictions should remain the same for WT and both mutant alleles. • Add caveat: original variant should be pathogenic according to the (modified) ACMG guidelines for variant classification	None
PS2 PM6_strong	*De novo* (paternity and maternity confirmed) in a patient with the disease and no family history. • Use the SVI WG point-based system • Multiple observations of de novo occurrence	Disease-specific

	• No family history: the EP recommends that the examination in the parents include an echocardiogram and full clinical work-up.
	• Phenotypic definitions:
	  ○ Highly specific for disease: TAAD + ectopia lentis
	  ○ Consistent with gene but not highly specific: TAAD + systemic score ≥7
	  ○ Consistent with gene but not highly specific or genetic heterogeneity: (isolated) TAAD, isolated ectopia lentis and in case of child (age <20yrs) systemic score >7	

PS3	• Well-established *in vitro* or *in vivo* functional studies supportive of a damaging effect. Use the 'Funtional Assay SVI Documentation' (https://clinicalgenome.org/working-groups/sequence-variant-interpretation/)
	For step 2 assessment
	• Functional studies deemed appropriate:
	  ○ cDNA analyses showing altered *FBN1* sequence
	  ○ Functional studies showing altered *FBN1* protein or RNA expression, proteolysis, folding, assembly, trafficking, secretion, Ca^{2+} binding, matrix deposition (cfr Dave Hollister assay), microfibril fragmentation/catabolism in an *in vitro* engineered system
	• Functional studies NOT deemed appropriate: non-specific altered TGF-beta signaling or histological hallmarks of medial degeneration, which are associated with many other types of variants in genes that are associated with MFS or HTAAD in general
	For step 3 assessment: Studies should be performed in the presence of NMD inhibitor.
		Disease-specific

PS4	The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls. *Each additional proband meeting Ghent criteria or having ectopia lentis may receive 1 point while those having only thoracic aortic disease or high systemic score or those for which the phenotype is not described in the literature will receive 0.5 points*	Disease-specific

	• If ≥ 4 points • Add caveat: BA1/BS1 should not be met	
PP1_strong	Co-segregation with disease in multiple affected family members • ≥5 affected individuals • Only count affected individuals from the same family (minus proband) that carry the variant or obligate carriers known with the disease • The EP will not specifically define "affected", "clinical examination" or cut-offs for aortic Z-scores. These are left to the discretion of the referring physicians.	Strength
PM1_strong	Located in a mutational hot spot and/or critical and well-established functional domain • Cysteine residues in cbEGF-like domains • Add caveat: PM5/PS1 should not be used when this argument applies.	Strength
MODERATE CRITERIA		
PM1	Located in a mutational hot spot and/or critical and well-established functional domain. • Cys in EGF-like domain, Cys in TB domain, Cys in hybrid domain, **(D/N)**-X-**(D/N)**-**(E/Q)**-Xm-**(D/N)**-Xn-**(Y/F)** substitution in cbEGF-like domain, invariant calcium-binding or hydroxylation residue in cbEGF-like domain, critical Gly between Cys2-Cys3 in cbEGF-like domain, Gly between Cys3-Cys4 if there is an upstream cbEGF domain, Cys-creating variants • Add caveat: N to S substitution in the second N of de consensus sequence and G to A might be tolerated, PM1 should not be used in these cases.	Disease-specific
PM2	Absent/rare from controls in an ethnically-matched cohort population sample.	N/A
PM3	For recessive disorders, detected in trans with a pathogenic variant.	N/A
PM4	Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants. • Add caveat: cannot be applied simultaneously with PVS1 (at any strength level)	None

PM5	Missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before. • Add caveat: Use argument with caution when the original missense variant created a cysteine especially in a cbEGF-like domain (cfr PM1_strong) as this may increase the pathogenicity level of this variant improperly. • Add caveat: original variant should be pathogenic according to the (modified) ACMG guidelines for variant classification	None
PM6 PS2_moderate	Confirmed de novo without confirmation of paternity and maternity. • Use the SVI WG point-based system • For further definition see PS2	Disease-specific
PVS1_moderate	Null variant in a gene where loss of function is a known mechanism of disease. • Follow the flowcharts, per SVI working group recommendation • In practice: ○ Initiation codon variant with 1 or more pathogenic variant(s) upstream of closest potential in-frame start codon	Strength
PS3_moderate	Well-established *in vitro* or *in vivo* functional studies supportive of a damaging effect. Use the 'Funtional Assay SVI Documentation'. For more details check PS3.	Strength
PS4_moderate	The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls. *Each additional proband meeting Ghent criteria or having ectopia lentis may receive 1 point while those having only thoracic aortic disease or high systemic score or those for which the phenotype is not described in the literature will receive 0.5 points* • If 2-3.5 points • Add caveat: BA1/BS1 should NOT be met	Strength
PP1_moderate	Co-segregation with disease in multiple affected family members • 4 affected individuals • Only count affected individuals from the same family (minus proband) that carry the variant or obligate carriers known with the disease	Strength

	• The EP will not specifically define "affected", "clinical examination" or cut-offs for aortic Z-scores. These are left to the discretion of the referring physicians.	
SUPPORTING CRITERIA		
PP1	Co-segregation with disease in multiple affected family members • 2-3 affected individuals • Only count affected individuals from the same family (minus proband) that carry the variant or obligate carriers known with the disease • The EP will not specifically define "affected", "clinical examination" or cut-offs for aortic Z-scores. These are left to the discretion of the referring physicians.	Disease-specific
PP2	Missense variant in a gene that has a low rate of benign missense variation and where missense variants are a common mechanism of disease. • Add caveat: if this argument is used pro-pathogenicity, there must be other arguments supporting pathogenicity, and no arguments supporting a benign assertion	None
PP3	Multiple lines of computational evidence support a deleterious effect on the gene or gene product • Recommended prediction program for missense variants: REVEL. Use 0.75 as a discriminatory cut-off value. • Recommended prediction programs for splice variants: GeneSplicer, MaxEntscan, and NNSPLICE. The outcome of all 3 prediction programs need to be in concordance.	Disease-specific
PP4	Phenotype specific for disease with single genetic etiology. • Use if patient fulfils revised Ghent criteria • Can be used if any of the family members have a highly specific phenotype	Disease-specific
PP5	Reputable source recently reports variant as pathogenic but the evidence is not available to the laboratory to perform an independent evaluation	*N/A*
PS3_supportive	Well-established *in vitro* or *in vivo* functional studies supportive of a damaging effect. Use the 'Funtional Assay SVI Documentation'. For more details check PS3.	Strength

PS4_supportive	The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls. *Each additional proband meeting Ghent criteria or having ectopia lentis may receive 1 point while those having only thoracic aortic disease or high systemic score or those for which the phenotype is not described in the literature will receive 0.5 point* • If *1-1.5 points* • Add caveat: BA1/BS1 should NOT be met	Strength
PM6_supportive PS2_supportive	Confirmed de novo without confirmation of paternity and maternity. • Use the SVI WG point-based system • For further definition see PS2	Strength
PM2_supportive	Absent/rare from controls in an ethnically-matched cohort population sample. • Threshold: <5.0E-6 (<0.0005%) • Use the highest ethnic population allele frequency • Caveat: PVS1 + PM2_Supportive may reach Likely Pathogenic • Caveat: Do not use Finnish, Ashkenazi Jewish, or "Other" populations in gnomAD. • Minimum amount of studied alleles should be 2000	Strength

BENIGN CRITERIA		
Criteria	**Criteria Description**	**Specification**
STAND ALONE CRITERIA		
BA1	Allele frequency above 0.1% in ExAc and gnomAD • Use the ethnic population with the highest allele frequency • Caveat: Do not use Finnish, Ashkenazi Jewish, or "Other" populations in gnomAD. • Minimum amount of studied alleles should be 2000	Disease-specific
STRONG CRITERIA		
BS1	Allele frequency greater than expected for disease (>0.005%) • Use the ethnic population with the highest allele frequency. • Caveat: Do not use Finnish, Ashkenazi Jewish, or "Other" populations in gnomAD. • Minimum amount of studied alleles should be 2000	Disease-specific
BS2	Observed in the homozygous state in a healthy adult	N/A

BS3	Well-established in vitro or in vivo functional studies shows no damaging effect on protein function • See PS3 for guidelines functional studies	None
BS4	Lack of segregation in affected members of a family. • Caution is warranted when the phenotype is not highly specific. Lack of segregation should then be clear in >1 affected family member	None
SUPPORTING CRITERIA		
BP1	Missense variant in gene where only LOF causes disease	N/A
BP2	Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder; or observed in cis with a pathogenic variant in any inheritance pattern. • Observed in trans in multiple cases (+2) with co-occuring pathogenic variants and phenotype is not more severe than when seen in isolation. • Observed in cis with a pathogenic variant, if the pathogenic variant has been seen in isolation in a patient with the disease phenotype	Disease-specific
BP3	In-frame deletions/insertions in a repetitive region without a known function	N/A
BP4	Multiple lines of computational evidence suggest no impact on gene or gene product • Recommended prediction program for missense variants: REVEL. Use 0.326 as a discriminatory cut-off value • Recommended prediction programs for splice variants: GeneSplicer, MaxEntscan, and NNSPLICE. The outcome of all 3 prediction programs need to be in concordance.	Disease-specific
BP5	Variant found in a case with an alternate molecular basis for disease	None
BP6	*Reputable source recently reports variant as benign but the evidence is not available to the laboratory to perform an independent evaluation*	*N/A*
BP7	A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved.	None

Key: **Disease-Specific:** Disease-specific modifications based on what is known about (gene); **Strength:** Increasing or decreasing strength of criteria based on the amount of evidence; **N/A:** not applicable for (gene); **None:** no changes made to existing criteria definitions.

VERY STRONG EVIDENCE OF PATHOGENICITY

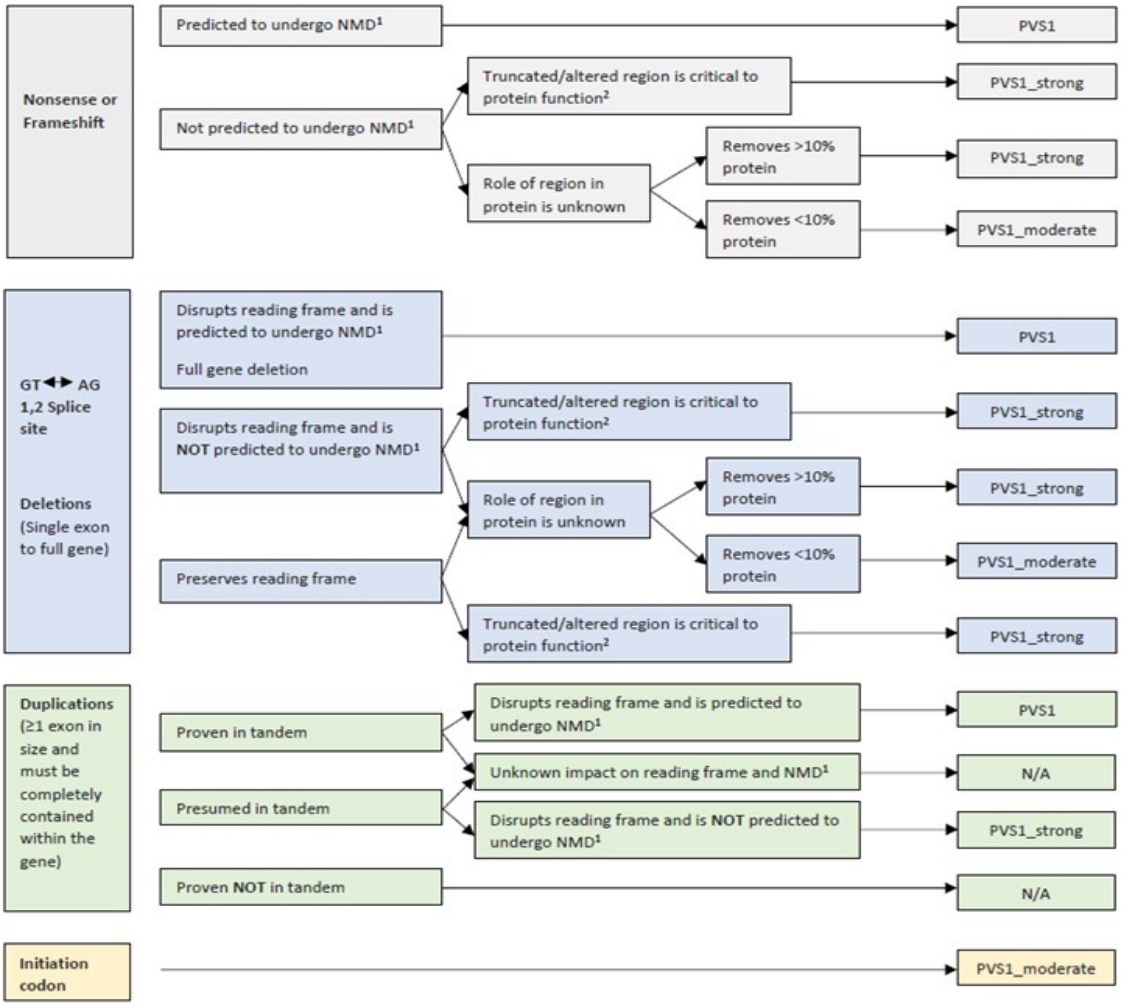
PVS1

Null variant (nonsense, frameshift, canonical +/-1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease

Caveats:

- Use caution interpreting LOF variants at the extreme 3' end of a gene
- Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact

FBN1 VCEP: PVS1 is applicable as described, but the panel has simplified the flowchart based on those arguments which only apply to *FBN1.*



Biological relevant FBN1 transcript is NM_00138

[1]NMD (Nonsense mediated mRNA decay) is predicted to occur when a stopcodon is integrated in the FBN1 sequence except for stopcodons in the last exon or the last 55 nucleotides of the penultimate exon

[2]Critical region: Use the same regions defined for the PM1 and PM1-strong arguments

PS2_very strong De novo (paternity and maternity confirmed) in a patient with the disease and no family history

FBN1 VCEP: Use the SVI WG point-based system (https://clinicalgenome.org/working-groups/sequence-variant-interpretation/)

The expert panel provided definitions for:

- 'highly specific phenotype': TAAD + ectopia lentis (mainly caused by variants in FBN1).
- 'consistent phenotype:' TAAD + systemic score ≥7 (can be caused by variants in few other HTAAD genes).
- 'consistent but genetic heterogeneity':(isolated) TAAD, isolated ectopia lentis and in case of a child (age <20yrs) systemic score≥ 7 in whom TAAD is progressive and can be developed later in life.

The EP recommends that the parents had a full clinical work-up and an echocardiogram to exclude MFS/HTAAD.

STRONG EVIDENCE OF PATHOGENICITY

PS1 Same amino acid change as a previously established pathogenic variant regardless of nucleotide change

FBN1 VCEP: PS1 is applicable as described. The EP wishes to include an extra caveats ((1) beware of changes that impact splicing rather than the amino acid. Splicing predictions should remain the same for WT and both mutant alleles; (2) the original variant should be pathogenic as defined by the (modified) ACMG guidelines for variant classification).

PS2 De novo (paternity and maternity confirmed) in a patient with the disease and no family history

FBN1 VCEP: Use the SVI WG point-based system.

The expert panel provided definitions for:

- 'highly specific phenotype': TAAD + ectopia lentis (mainly caused by variants in FBN1).
- 'consistent phenotype:' TAAD + systemic score ≥7 (can be caused by variants in few other HTAAD genes).
- 'consistent but genetic heterogeneity':(isolated) TAAD, isolated ectopia lentis and in case of a child (age <20yrs) systemic score≥ 7 in whom TAAD is progressive and can be developed later in life.

The EP recommends that the parents had a full clinical work-up and an echocardiogram to exclude MFS/HTAAD.

PS3 Well-established *in vitro* or *in vivo* functional studies supportive of a damaging effect.

The FBN1 VCEP recommends to use the 'Funtional Assay SVI Documentation' (https://clinicalgenome.org/working-groups/sequence-variant-interpretation/)

For step 2 assessment:

- Functional studies deemed appropriate:
  - cDNA analyses showing altered *FBN1* sequence.
  - Functional studies showing altered *FBN1* protein or RNA expression, proteolysis, folding, assembly, trafficking, secretion, Ca^{2+} binding, matrix deposition (cfr Dave Hollister assay), microfibril fragmentation/catabolism in an *in vitro* engineered system.
- Functional studies NOT deemed appropriate: non-specific altered TGF-beta signaling or histological hallmarks of medial degeneration, which are associated with many other types of variants in genes that are associated with MFS or HTAAD in general.

For step 3 assessment: Studies should be performed in the presence of NMD inhibitor.

PS4 The prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls

FBN1 VCEP: Each additional proband meeting Ghent criteria or having ectopia lentis may receive 1 point while those having only thoracic aortic disease or high systemic score or those for which the phenotype is not described in the literature will receive 0.5 point. PS4 is applicable if ≥4 points are met with the caveat that BA1/BS1 should not be met.

PVS1_strong Null variant (nonsense, frameshift, canonical +/-1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease

FBN1 VCEP: PVS1_strong is applicable as described in the flowchart.

PP1_strong Co-segregation with disease in multiple affected family members

FBN1 VCEP: Use PP1 as strong argument if

- ≥5 affected individuals
- Only count affected individuals (minus proband) that carry the variant or obligate carriers known with the disease.
- The EP will not specifically define “affected”, “clinical examination” or cut-offs for aortic Z-scores. These are left to the discretion of the referring physicians.

Note 1: caution needed when counting segregations in presence of other possible disease-causing variants.

Note 2: caution needed when distantly related affected individuals are connected by unknown or unaffected relatives (raises possibility of multiple causes of disease).

PM1_strong Located in a mutational hot spot and/or critical and well-established functional domain
FBN1 VCEP: substitution of cysteine residues in cbEGF-like domains is considered very likely to be pathogenic given literature-based evidence. A table with the residues for which PM1_strong could be considered can be found as supplemental material.
Note 1: whenever this argument applies, PM5/PS1 should not be used.

MODERATE EVIDENCE OF PATHOGENICITY

PM1 Located in a mutational hot spot and/or critical and well-established functional domain (*e.g.* active site of an enzyme) without benign variation

- Cys in EGF-like domain, Cys in TB domain, Cys in hybrid domain, **(D/N)**-X-**(D/N)**-**(E/Q)**-Xm-**(D/N)**-Xn-**(Y/F)** substitution in cbEGF-like domain, invariant calcium-binding or hydroxylation residue in cbEGF-like domain, critical Gly between Cys2-Cys3 in cbEGF-like domain, Gly between Cys3-Cys4 if there is an upstream cbEGF domain, Cys-creating variants.
- Add caveat: N to S substitution in the second N of de consensus sequence and G to A might be tolerated, PM1 should not be used in these cases.

A table with the residues for which PM1 could be considered can be found as supplemental material.

PM2 Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes or ExAC
FBN1 VCEP: not applicable.

PM3 For recessive disorders, detected in *trans* with a pathogenic variant
Note: This requires testing of parents (or offspring) to determine phase
FBN1 VCEP: not applicable.

PM4 Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants

FBN1 VCEP: PM4 is applicable as described. The EP added a caveat (cannot be applied simultaneously with PVS1 (any strength level).

PM5 Missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before

FBN1 VCEP: PM5 is applicable as described. The EP added a caveat (Use argument with caution when the original missense variant created a cysteine especially in a cbEGF-like domain (cfr PM1) as this may increase the pathogenicity level of this variant improperly).

PM6 Assumed *de novo*, but without confirmation of paternity and maternity

FBN1 VCEP: Use the SVI WG point-based system.

The expert panel provided definitions for:

- 'highly specific phenotype':TAAD + ectopia lentis (mainly caused by variants in FBN1).
- 'consistent phenotype:' TAAD + systemic score ≥7 (can be caused by variants in few other HTAAD genes).
- 'consistent but genetic heterogeneity':(isolated) TAAD, isolated ectopia lentis and in case of a child (age <20yrs) systemic score≥ 7 in whom TAAD is progressive and can be developed later in life.

The EP recommends that the parents had a full clinical work-up and an echocardiogram to exclude MFS/HTAAD.

PVS1_moderate Null variant (nonsense, frameshift, canonical +/-1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease

FBN1 VCEP: PVS1_moderate is applicable as described.

PS3_moderate Well-established *in vitro* or *in vivo* functional studies supportive of a damaging effect on the gene or gene product

FBN1 VCEP: Follow the 'Funtional Assay SVI Documentation'.

PS4_moderate The prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls

FBN1 VCEP: Each additional proband meeting Ghent criteria or having ectopia lentis may receive 1 point while those having only thoracic aortic disease or high systemic score or those for which the phenotype is not described in the literature will receive 0.5 point. PS4_moderate is applicable if 2-3.5 points are met with the caveat that that BA1/BS1 should not be met.

PP1_moderate Co-segregation with disease in multiple affected family members.
FBN1 VCEP:

- 4 affected individuals.
- Only count affected individuals (minus proband) that carry the variant or obligate carriers known with the disease.
- The EP will not specifically define "unaffected", "clinical examination" or cut-offs for aortic Z-scores. These are left to the discretion of the referring physicians.

Check PP1_strong for specific notes of caution.

SUPPORTING EVIDENCE OF PATHOGENICITY

PP1 Co-segregation with disease in multiple affected family members in a gene definitively
known to cause the disease
FBN1 VCEP:

- 2-3 affected individuals.
- Only count affected individuals (minus proband) that carry the variant or obligate carriers known with the disease.
- The EP will not specifically define "affected", "clinical examination" or cut-offs for aortic Z-scores. These are left to the discretion of the referring physicians.

Check PP1_strong for specific notes of caution.

PP2 Missense variant in a gene that has a low rate of benign missense variation and where missense variants are a common mechanism of disease
FBN1 VCEP: Based on the SVI WG recommendation concerning constraint scores: if missense constraint z scores are > 3.09 in ExAC, this criterion may be used. For *FBN1*, the constraint score is higher than 5. Hence, PP2 is applicable as described. However, the EP wishes to include a caveat (if this argument is used pro-pathogenicity, there must be other arguments supporting pathogenicity, and no arguments supporting a benign assertion).

PP3 Multiple lines of computational evidence support a deleterious effect on the gene or
gene product (conservation, evolutionary, splicing impact, etc)
FBN1 VCEP: PP3 is applicable as described. Prediction programs of choice:

- For missense variants REVEL. Use 0.75 as discriminatory cut-off value

- For splice-site variables GeneSplicer, MaxEntScan and NNSPLICE (splice site). All three programs should be in concordance.

PP4 Patient's phenotype or family history is highly specific for a disease with a single genetic etiology.

FBN1 VCEP:

- Use if patients fulfills the revised Ghent criteria
- Can be used if any of the family members have a highly specific phenotype.

PP5 Reputable source recently reports variant as pathogenic but the evidence is not available
to the laboratory to perform an independent evaluation

FBN1 VCEP: Not applicable.

PVS1_sup Null variant (nonsense, frameshift, canonical +/-1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease

FBN1 VCEP: PVS1_supportive is applicable as described.

PS4_sup The prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls

FBN1 VCEP: Each additional proband meeting Ghent criteria or having ectopia lentis may receive 1 point while those having only thoracic aortic disease or high systemic score or those for which the phenotype is not described in the literature will receive 0.5 point. PS4_supportive is applicable if 1-1.5 points are met with the caveat that that BA1/BS1 should not be met.

PM2_sup Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes or ExAC

FBN1 VCEP: threshold was set to <0.0005%

Note 1: Use highest ethnic allele frequency of a variant to evaluate PM2 with the following caveats:

- *Minimum of studied alleles should be 2000.*
- *Do not use Finnish, Ashkenazi Jewish, or "Other" populations in gnomAD.*

FBN1 VCEP: PVS1 + PM2_sup may reach a likely pathogenic variant as per recommendation of the SVI.

PM6_sup Assumed *de novo*, but without confirmation of paternity and maternity

FBN1 VCEP: Use the SVI WG point-based system.

The expert panel provided definitions for:

- 'highly specific phenotype':TAAD + ectopia lentis (mainly caused by variants in FBN1).
- 'consistent phenotype:' TAAD + systemic score ≥7 (can be caused by variants in few other HTAAD genes).
- 'consistent but genetic heterogeneity':(isolated) TAAD, isolated ectopia lentis and in case of a child (age <20yrs) systemic score≥ 7 in whom TAAD is progressive and can be developed later in life.

The EP recommends that the parents had a full clinical work-up and an echocardiogram to exclude MFS/HTAAD.

STAND ALONE EVIDENCE OF BENIGN IMPACT

BA1 Allele frequency is above 5% in Exome Sequencing Project, 1000 Genomes, or ExAC

FBN1 VCEP: new threshold is set at 0.1%.

Note 1: Use highest ethnic allele frequency of a variant to evaluate PM2

- *Minimum of studied alleles should be 2000.*
- *Do not use Finnish, Ashkenazi Jewish, or "Other" populations in gnomAD.*

STRONG EVIDENCE OF BENIGN IMPACT

BS1 Allele frequency is greater than expected for disorder

FBN1 VCEP: MAF changed to a frequency between 0.1% and 0.005%.

Note 1: Use highest ethnic allele frequency of a variant to evaluate PM2

- *Minimum of studied alleles should be 2000.*
- *Do not use Finnish, Ashkenazi Jewish, or "Other" populations in gnomAD.*

BS2 Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder with full penetrance expected at an early age.

FBN1 VCEP: not applicable.

BS3 Well-established *in vitro* or *in vivo* functional studies show no damaging effect on protein function or splicing.

FBN1 VCEP: see PS3 for guidelines functional studies.

BS4 Lack of segregation in affected members of a family
FBN1 VCEP: BS4 is applicable as described. The EP wants to add a word of caution in case the phenotype is not highly specific. Lack of segregation should then be identified in >1 affected family member.

SUPPORTING EVIDENCE FOR BENIGN IMPACT

BP1 Missense variant in a gene for which primarily truncating variants are known to cause disease
FBN1 VCEP: not applicable.

BP2 Observed in *trans* with a pathogenic variant for a fully penetrant dominant gene/disorder; or observed in *cis* with a pathogenic variant in any inheritance pattern
FBN1 VCEP:

- Observed in trans in multiple cases (+2) with co-occurring pathogenic variants and phenotype is not more severe than when seen in isolation.
- Observed in cis with a pathogenic variant, if the pathogenic variant has been seen in isolation in a patient with the disease phenotype.

BP3 In-frame deletions/insertions in a repetitive region without a known function
FBN1 VCEP: not applicable.

BP4 Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.)

- Recommended prediction program for missense variants: REVEL. Use 0.326 as a discriminatory cut-off value.
- Recommended prediction programs for splice-site variants: GeneSplicer, MaxEntscan and NNSPLICE. The outcome of all 3 prediction programmed need to be in concordance.

BP5 Variant found in a case with an alternate molecular basis for disease
FBN1 VCEP: BP5 applicable as described.

BP6 Reputable source recently reports variant as benign but the evidence is not available to the laboratory to perform an independent evaluation
FBN1 VCEP: not applicable.

BP7 A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved
FBN1 VCEP: BP7 applicable as described.

RULES FOR COMBINING PATHOGENIC CRITERIA

Pathogenic Very Strong: PVS1, PS2_very strong, PM6_very strong

Pathogenic Strong: PS1-PS4, PVS1_strong, PM1_strong, PM6_strong, PP1_strong

Pathogenic Moderate: PM1-PM6, PVS1_moderate, PS2_moderate, PS3_moderate, PP1_moderate

Pathogenic Supporting: PP1-PP4, PVS1_supportive, PS2_supportive, PM2_supportive, PM6_supportive

Benign stand alone: BA1

Benign strong: BS1-BS4

Benign supporting: BP1-BP7

Pathogenic

1. 1 Very Strong AND
    a. ≥1 Strong OR
    b. ≥2 Moderate OR
    c. 1 Moderate and 1 Supporting OR
    d. ≥2 Supporting
2. ≥2 Strong OR
3. 1 Strong AND
    a. ≥3 Moderate OR
    b. 2 Moderate AND ≥2 Supporting OR
    c. 1 Moderate AND ≥4 Supporting

Likely Pathogenic

1. 1 Very Strong AND 1 Moderate OR
2. 1 Very strong AND PM2_supportive OR
3. 1 Strong AND 1-2 Moderate OR
4. 1 Strong AND ≥2 Supporting OR
5. ≥3 Moderate OR
6. 2 Moderate AND ≥2 Supporting OR
7. 1 Moderate AND ≥4 Supporting

RULES FOR COMBINING BENIGN CRITERIA

Benign

1. 1 Stand-Alone OR
2. ≥2 Strong

Likely Benign

1. 1 Strong and 1 Supporting OR
2. ≥2 Supporting

SUPPLEMENTAL MATERIAL:

Residues for PM1_strong and PM1 consideration

Related publication(s): **Date Approved:** January 4, 2022
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ClinGen_FBN1_ACMG_Specifications_v1

Residues for PM1_strong consideration				
Exon(s)	**Domain**	**Residue**	**Structure analysis**	**Potential impact**
7	cbEGF1	p.Cys250	disulfide bond	folding defect
7	cbEGF1	p.Cys257	disulfide bond	folding defect
7	cbEGF1	p.Cys262	disulfide bond	folding defect
7	cbEGF1	p.Cys271	disulfide bond	folding defect
7	cbEGF1	p.Cys273	disulfide bond	folding defect
7	cbEGF1	p.Cys286	disulfide bond	folding defect
8	cbEGF2	p.Cys292	disulfide bond	folding defect
8	cbEGF2	p.Cys299	disulfide bond	folding defect
8	cbEGF2	p.Cys304	disulfide bond	folding defect
8	cbEGF2	p.Cys313	disulfide bond	folding defect
8	cbEGF2	p.Cys315	disulfide bond	folding defect
8	cbEGF2	p.Cys328	disulfide bond	folding defect
12	cbEGF3	p.Cys494	disulfide bond	folding defect
12	cbEGF3	p.Cys499	disulfide bond	folding defect
12	cbEGF3	p.Cys504	disulfide bond	folding defect
12	cbEGF3	p.Cys513	disulfide bond	folding defect
12	cbEGF3	p.Cys515	disulfide bond	folding defect
12	cbEGF3	p.Cys528	disulfide bond	folding defect
13	cbEGF4	p.Cys534	disulfide bond	folding defect
13	cbEGF4	p.Cys541	disulfide bond	folding defect
13	cbEGF4	p.Cys546	disulfide bond	folding defect
13	cbEGF4	p.Cys555	disulfide bond	folding defect
13	cbEGF4	p.Cys557	disulfide bond	folding defect
13	cbEGF4	p.Cys570	disulfide bond	folding defect
14	cbEGF5	p.Cys576	disulfide bond	folding defect
14	cbEGF5	p.Cys582	disulfide bond	folding defect
14	cbEGF5	p.Cys587	disulfide bond	folding defect
14	cbEGF5	p.Cys596	disulfide bond	folding defect
14	cbEGF5	p.Cys598	disulfide bond	folding defect
14	cbEGF5	p.Cys611	disulfide bond	folding defect
15	cbEGF6	p.Cys617	disulfide bond	folding defect
15	cbEGF6	p.Cys623	disulfide bond	folding defect
15	cbEGF6	p.Cys628	disulfide bond	folding defect
15	cbEGF6	p.Cys637	disulfide bond	folding defect
15	cbEGF6	p.Cys639	disulfide bond	folding defect
15	cbEGF6	p.Cys652	disulfide bond	folding defect
18	cbEGF7	p.Cys727	disulfide bond	folding defect
18	cbEGF7	p.Cys734	disulfide bond	folding defect
18	cbEGF7	p.Cys739	disulfide bond	folding defect
18	cbEGF7	p.Cys748	disulfide bond	folding defect
18	cbEGF7	p.Cys750	disulfide bond	folding defect
18	cbEGF7	p.Cys763	disulfide bond	folding defect
19	cbEGF8	p.Cys769	disulfide bond	folding defect
19	cbEGF8	p.Cys776	disulfide bond	folding defect
19	cbEGF8	p.Cys781	disulfide bond	folding defect

19	cbEGF8	p.Cys790	disulfide bond	folding defect
19	cbEGF8	p.Cys792	disulfide bond	folding defect
19	cbEGF8	p.Cys805	disulfide bond	folding defect
20	cbEGF9	p.Cys811	disulfide bond	folding defect
20	cbEGF9	p.Cys816	disulfide bond	folding defect
20	cbEGF9	p.Cys821	disulfide bond	folding defect
20	cbEGF9	p.Cys830	disulfide bond	folding defect
20	cbEGF9	p.Cys832	disulfide bond	folding defect
20	cbEGF9	p.Cys845	disulfide bond	folding defect
23	cbEGF10	p.Cys914	disulfide bond	folding defect
23	cbEGF10	p.Cys921	disulfide bond	folding defect
23	cbEGF10	p.Cys926	disulfide bond	folding defect
23	cbEGF10	p.Cys935	disulfide bond	folding defect
23	cbEGF10	p.Cys937	disulfide bond	folding defect
23	cbEGF10	p.Cys950	disulfide bond	folding defect
25	cbEGF11	p.Cys1032	disulfide bond	folding defect
25	cbEGF11	p.Cys1039	disulfide bond	folding defect
25	cbEGF11	p.Cys1044	disulfide bond	folding defect
25	cbEGF11	p.Cys1053	disulfide bond	folding defect
25	cbEGF11	p.Cys1055	disulfide bond	folding defect
25	cbEGF11	p.Cys1068	disulfide bond	folding defect
26	cbEGF12	p.Cys1074	disulfide bond	folding defect
26	cbEGF12	p.Cys1081	disulfide bond	folding defect
26	cbEGF12	p.Cys1086	disulfide bond	folding defect
26	cbEGF12	p.Cys1095	disulfide bond	folding defect
26	cbEGF12	p.Cys1097	disulfide bond	folding defect
26	cbEGF12	p.Cys1111	disulfide bond	folding defect
27	cbEGF13	p.Cys1117	disulfide bond	folding defect
27	cbEGF13	p.Cys1124	disulfide bond	folding defect
27	cbEGF13	p.Cys1129	disulfide bond	folding defect
27	cbEGF13	p.Cys1138	disulfide bond	folding defect
27	cbEGF13	p.Cys1140	disulfide bond	folding defect
27	cbEGF13	p.Cys1153	disulfide bond	folding defect
28	cbEGF14	p.Cys1159	disulfide bond	folding defect
28	cbEGF14	p.Cys1166	disulfide bond	folding defect
28	cbEGF14	p.Cys1171	disulfide bond	folding defect
28	cbEGF14	p.Cys1180	disulfide bond	folding defect
28	cbEGF14	p.Cys1182	disulfide bond	folding defect
28	cbEGF14	p.Cys1195	disulfide bond	folding defect
29	cbEGF15	p.Cys1201	disulfide bond	folding defect
29	cbEGF15	p.Cys1208	disulfide bond	folding defect
29	cbEGF15	p.Cys1212	disulfide bond	folding defect
29	cbEGF15	p.Cys1221	disulfide bond	folding defect
29	cbEGF15	p.Cys1223	disulfide bond	folding defect
29	cbEGF15	p.Cys1236	disulfide bond	folding defect
30	cbEGF16	p.Cys1242	disulfide bond	folding defect
30	cbEGF16	p.Cys1249	disulfide bond	folding defect
30	cbEGF16	p.Cys1254	disulfide bond	folding defect

30	cbEGF16	p.Cys1263	disulfide bond	folding defect
30	cbEGF16	p.Cys1265	disulfide bond	folding defect
30	cbEGF16	p.Cys1278	disulfide bond	folding defect
31	cbEGF17	p.Cys1284	disulfide bond	folding defect
31	cbEGF17	p.Cys1291	disulfide bond	folding defect
31	cbEGF17	p.Cys1296	disulfide bond	folding defect
31	cbEGF17	p.Cys1305	disulfide bond	folding defect
31	cbEGF17	p.Cys1307	disulfide bond	folding defect
31	cbEGF17	p.Cys1320	disulfide bond	folding defect
32	cbEGF18	p.Cys1326	disulfide bond	folding defect
32	cbEGF18	p.Cys1333	disulfide bond	folding defect
32	cbEGF18	p.Cys1339	disulfide bond	folding defect
32	cbEGF18	p.Cys1348	disulfide bond	folding defect
32	cbEGF18	p.Cys1350	disulfide bond	folding defect
32	cbEGF18	p.Cys1361	disulfide bond	folding defect
33	cbEGF19	p.Cys1367	disulfide bond	folding defect
33	cbEGF19	p.Cys1374	disulfide bond	folding defect
33	cbEGF19	p.Cys1380	disulfide bond	folding defect
33	cbEGF19	p.Cys1389	disulfide bond	folding defect
33	cbEGF19	p.Cys1391	disulfide bond	folding defect
33	cbEGF19	p.Cys1402	disulfide bond	folding defect
34	cbEGF20	p.Cys1408	disulfide bond	folding defect
34	cbEGF20	p.Cys1415	disulfide bond	folding defect
34	cbEGF20	p.Cys1420	disulfide bond	folding defect
34	cbEGF20	p.Cys1429	disulfide bond	folding defect
34	cbEGF20	p.Cys1431	disulfide bond	folding defect
34	cbEGF20	p.Cys1444	disulfide bond	folding defect
35	cbEGF21	p.Cys1450	disulfide bond	folding defect
35	cbEGF21	p.Cys1456	disulfide bond	folding defect
35	cbEGF21	p.Cys1461	disulfide bond	folding defect
35	cbEGF21	p.Cys1470	disulfide bond	folding defect
35	cbEGF21	p.Cys1472	disulfide bond	folding defect
35	cbEGF21	p.Cys1485	disulfide bond	folding defect
36	cbEGF22	p.Cys1491	disulfide bond	folding defect
36	cbEGF22	p.Cys1497	disulfide bond	folding defect
36	cbEGF22	p.Cys1502	disulfide bond	folding defect
36	cbEGF22	p.Cys1511	disulfide bond	folding defect
36	cbEGF22	p.Cys1513	disulfide bond	folding defect
36	cbEGF22	p.Cys1526	disulfide bond	folding defect
39	cbEGF23	p.Cys1610	disulfide bond	folding defect
39	cbEGF23	p.Cys1617	disulfide bond	folding defect
39	cbEGF23	p.Cys1622	disulfide bond	folding defect
39	cbEGF23	p.Cys1631	disulfide bond	folding defect
39	cbEGF23	p.Cys1633	disulfide bond	folding defect
39	cbEGF23	p.Cys1646	disulfide bond	folding defect
40	cbEGF24	p.Cys1652	disulfide bond	folding defect
40	cbEGF24	p.Cys1658	disulfide bond	folding defect
40	cbEGF24	p.Cys1663	disulfide bond	folding defect

40	cbEGF24	p.Cys1672	disulfide bond	folding defect
40	cbEGF24	p.Cys1674	disulfide bond	folding defect
40	cbEGF24	p.Cys1687	disulfide bond	folding defect
43	cbEGF25	p.Cys1770	disulfide bond	folding defect
43	cbEGF25	p.Cys1777	disulfide bond	folding defect
43	cbEGF25	p.Cys1782	disulfide bond	folding defect
43	cbEGF25	p.Cys1791	disulfide bond	folding defect
43	cbEGF25	p.Cys1793	disulfide bond	folding defect
43	cbEGF25	p.Cys1806	disulfide bond	folding defect
44	cbEGF26	p.Cys1812	disulfide bond	folding defect
44	cbEGF26	p.Cys1818	disulfide bond	folding defect
44	cbEGF26	p.Cys1824	disulfide bond	folding defect
44	cbEGF26	p.Cys1833	disulfide bond	folding defect
44	cbEGF26	p.Cys1835	disulfide bond	folding defect
44	cbEGF26	p.Cys1847	disulfide bond	folding defect
45	cbEGF27	p.Cys1853	disulfide bond	folding defect
45	cbEGF27	p.Cys1860	disulfide bond	folding defect
45	cbEGF27	p.Cys1865	disulfide bond	folding defect
45	cbEGF27	p.Cys1874	disulfide bond	folding defect
45	cbEGF27	p.Cys1876	disulfide bond	folding defect
45	cbEGF27	p.Cys1889	disulfide bond	folding defect
46	cbEGF28	p.Cys1895	disulfide bond	folding defect
46	cbEGF28	p.Cys1900	disulfide bond	folding defect
46	cbEGF28	p.Cys1905	disulfide bond	folding defect
46	cbEGF28	p.Cys1914	disulfide bond	folding defect
46	cbEGF28	p.Cys1916	disulfide bond	folding defect
46	cbEGF28	p.Cys1928	disulfide bond	folding defect
47	cbEGF29	p.Cys1934	disulfide bond	folding defect
47	cbEGF29	p.Cys1942	disulfide bond	folding defect
47	cbEGF29	p.Cys1947	disulfide bond	folding defect
47	cbEGF29	p.Cys1956	disulfide bond	folding defect
47	cbEGF29	p.Cys1958	disulfide bond	folding defect
47	cbEGF29	p.Cys1971	disulfide bond	folding defect
48	cbEGF30	p.Cys1977	disulfide bond	folding defect
48	cbEGF30	p.Cys1984	disulfide bond	folding defect
48	cbEGF30	p.Cys1989	disulfide bond	folding defect
48	cbEGF30	p.Cys1998	disulfide bond	folding defect
48	cbEGF30	p.Cys2000	disulfide bond	folding defect
48	cbEGF30	p.Cys2011	disulfide bond	folding defect
49	cbEGF31	p.Cys2017	disulfide bond	folding defect
49	cbEGF31	p.Cys2024	disulfide bond	folding defect
49	cbEGF31	p.Cys2029	disulfide bond	folding defect
49	cbEGF31	p.Cys2038	disulfide bond	folding defect
49	cbEGF31	p.Cys2040	disulfide bond	folding defect
49	cbEGF31	p.Cys2053	disulfide bond	folding defect
52	cbEGF32	p.Cys2131	disulfide bond	folding defect
52	cbEGF32	p.Cys2137	disulfide bond	folding defect
52	cbEGF32	p.Cys2142	disulfide bond	folding defect

52	cbEGF32	p.Cys2151	disulfide bond	folding defect
52	cbEGF32	p.Cys2153	disulfide bond	folding defect
52	cbEGF32	p.Cys2164	disulfide bond	folding defect
53	cbEGF33	p.Cys2170	disulfide bond	folding defect
53	cbEGF33	p.Cys2176	disulfide bond	folding defect
53	cbEGF33	p.Cys2181	disulfide bond	folding defect
53	cbEGF33	p.Cys2190	disulfide bond	folding defect
53	cbEGF33	p.Cys2192	disulfide bond	folding defect
53	cbEGF33	p.Cys2204	disulfide bond	folding defect
54	cbEGF34	p.Cys2210	disulfide bond	folding defect
54	cbEGF34	p.Cys2217	disulfide bond	folding defect
54	cbEGF34	p.Cys2221	disulfide bond	folding defect
54	cbEGF34	p.Cys2230	disulfide bond	folding defect
54	cbEGF34	p.Cys2232	disulfide bond	folding defect
54	cbEGF34	p.Cys2245	disulfide bond	folding defect
55	cbEGF35	p.Cys2251	disulfide bond	folding defect
55	cbEGF35	p.Cys2258	disulfide bond	folding defect
55	cbEGF35	p.Cys2265	disulfide bond	folding defect
55	cbEGF35	p.Cys2274	disulfide bond	folding defect
55	cbEGF35	p.Cys2276	disulfide bond	folding defect
55	cbEGF35	p.Cys2289	disulfide bond	folding defect
56	cbEGF36	p.Cys2295	disulfide bond	folding defect
56	cbEGF36	p.Cys2302	disulfide bond	folding defect
56	cbEGF36	p.Cys2307	disulfide bond	folding defect
56	cbEGF36	p.Cys2316	disulfide bond	folding defect
56	cbEGF36	p.Cys2318	disulfide bond	folding defect
56	cbEGF36	p.Cys2331	disulfide bond	folding defect
58	cbEGF37	p.Cys2406	disulfide bond	folding defect
58	cbEGF37	p.Cys2413	disulfide bond	folding defect
58	cbEGF37	p.Cys2418	disulfide bond	folding defect
58	cbEGF37	p.Cys2427	disulfide bond	folding defect
58	cbEGF37	p.Cys2429	disulfide bond	folding defect
58	cbEGF37	p.Cys2442	disulfide bond	folding defect
59	cbEGF38	p.Cys2448	disulfide bond	folding defect
59	cbEGF38	p.Cys2455	disulfide bond	folding defect
59	cbEGF38	p.Cys2459	disulfide bond	folding defect
59	cbEGF38	p.Cys2468	disulfide bond	folding defect
59	cbEGF38	p.Cys2470	disulfide bond	folding defect
59	cbEGF38	p.Cys2483	disulfide bond	folding defect
60	cbEGF39	p.Cys2489	disulfide bond	folding defect
60	cbEGF39	p.Cys2496	disulfide bond	folding defect
60	cbEGF39	p.Cys2500	disulfide bond	folding defect
60	cbEGF39	p.Cys2509	disulfide bond	folding defect
60	cbEGF39	p.Cys2511	disulfide bond	folding defect
60	cbEGF39	p.Cys2522	disulfide bond	folding defect
61	cbEGF40	p.Cys2528	disulfide bond	folding defect
61	cbEGF40	p.Cys2535	disulfide bond	folding defect

61	cbEGF40	p.Cys2541	disulfide bond	folding defect
61	cbEGF40	p.Cys2550	disulfide bond	folding defect
61	cbEGF40	p.Cys2552	disulfide bond	folding defect
61	cbEGF40	p.Cys2565	disulfide bond	folding defect
62	cbEGF41	p.Cys2571	disulfide bond	folding defect
62	cbEGF41	p.Cys2577	disulfide bond	folding defect
62	cbEGF41	p.Cys2581	disulfide bond	folding defect
62	cbEGF41	p.Cys2590	disulfide bond	folding defect
62	cbEGF41	p.Cys2592	disulfide bond	folding defect
62	cbEGF41	p.Cys2605	disulfide bond	folding defect
63	cbEGF42	p.Cys2611	disulfide bond	folding defect
63	cbEGF42	p.Cys2617	disulfide bond	folding defect
63	cbEGF42	p.Cys2622	disulfide bond	folding defect
63	cbEGF42	p.Cys2631	disulfide bond	folding defect
63	cbEGF42	p.Cys2633	disulfide bond	folding defect
63	cbEGF42	p.Cys2646	disulfide bond	folding defect
63-64	cbEGF43	p.Cys2652	disulfide bond	folding defect
63-64	cbEGF43	p.Cys2659	disulfide bond	folding defect
63-64	cbEGF43	p.Cys2663	disulfide bond	folding defect
63-64	cbEGF43	p.Cys2672	disulfide bond	folding defect
63-64	cbEGF43	p.Cys2674	disulfide bond	folding defect
63-64	cbEGF43	p.Cys2686	disulfide bond	folding defect

Residues for PM1 consideration

2-3	EGF1	p.Cys85	disulfide bond	folding defect
2-3	EGF1	p.Cys89	disulfide bond	folding defect
2-3	EGF1	p.Cys94	disulfide bond	folding defect
2-3	EGF1	p.Cys100	disulfide bond	folding defect
2-3	EGF1	p.Cys102	disulfide bond	folding defect
2-3	EGF1	p.Cys111	disulfide bond	folding defect
3-4	EGF2	p.Cys119	disulfide bond	folding defect
3-4	EGF2	p.Cys123	disulfide bond	folding defect
3-4	EGF2	p.Cys129	disulfide bond	folding defect
3-4	EGF2	p.Cys134	disulfide bond	folding defect
3-4	EGF2	p.Cys136	disulfide bond	folding defect
3-4	EGF2	p.Cys145	disulfide bond	folding defect
4-5	EGF3	p.Cys150	disulfide bond	folding defect
4-5	EGF3	p.Cys154	disulfide bond	folding defect
4-5	EGF3	p.Cys160	disulfide bond	folding defect
4-5	EGF3	p.Cys166	disulfide bond	folding defect
4-5	EGF3	p.Cys168	disulfide bond	folding defect
4-5	EGF3	p.Cys177	disulfide bond	folding defect
5,6	Hyb1	p.Cys186	disulfide bond	folding defect
5,6	Hyb1	p.Cys195	disulfide bond	folding defect
5,6	Hyb1	p.Cys204	disulfide bond	folding defect
5,6	Hyb1	p.Cys209	disulfide bond	folding defect
5,6	Hyb1	p.Cys210	disulfide bond	folding defect
5,6	Hyb1	p.Cys221	disulfide bond	folding defect

5,6	Hyb1	p.Cys224	disulfide bond	folding defect
5,6	Hyb1	p.Cys231	disulfide bond	folding defect
5,6	Hyb1	p.Cys244	disulfide bond	folding defect
11	EGF2	p.Cys453	disulfide bond	folding defect
11	EGF2	p.Cys460	disulfide bond	folding defect
11	EGF2	p.Cys465	disulfide bond	folding defect
11	EGF2	p.Cys474	disulfide bond	folding defect
11	EGF2	p.Cys476	disulfide bond	folding defect
11	EGF2	p.Cys488	disulfide bond	folding defect
9-10	TB1	p.Cys336	disulfide bond	folding defect
9-10	TB1	p.Cys345	disulfide bond	folding defect
9-10	TB1	p.Cys358	disulfide bond	folding defect
9-10	TB1	p.Cys359	disulfide bond	folding defect
9-10	TB1	p.Cys360	disulfide bond	folding defect
9-10	TB1	p.Cys365	disulfide bond	folding defect
9-10	TB1	p.Cys377	disulfide bond	folding defect
9-10	TB1	p.Cys389	disulfide bond	folding defect
16-17	TB2	p.Cys661	disulfide bond	folding defect
16-17	TB2	p.Cys670	disulfide bond	folding defect
16-17	TB2	p.Cys683	disulfide bond	folding defect
16-17	TB2	p.Cys684	disulfide bond	folding defect
16-17	TB2	p.Cys685	disulfide bond	folding defect
16-17	TB2	p.Cys696	disulfide bond	folding defect
16-17	TB2	p.Cys699	disulfide bond	folding defect
16-17	TB2	p.Cys711	disulfide bond	folding defect
21-22	Hyb2	p.Cys853	disulfide bond	folding defect
21-22	Hyb2	p.Cys862	disulfide bond	folding defect
21-22	Hyb2	p.Cys875	disulfide bond	folding defect
21-22	Hyb2	p.Cys876	disulfide bond	folding defect
21-22	Hyb2	p.Cys887	disulfide bond	folding defect
21-22	Hyb2	p.Cys890	disulfide bond	folding defect
21-22	Hyb2	p.Cys896	disulfide bond	folding defect
24	TB3	p.Cys958	disulfide bond	folding defect
24	TB3	p.Cys967	disulfide bond	folding defect
24	TB3	p.Cys980	disulfide bond	folding defect
24	TB3	p.Cys981	disulfide bond	folding defect
24	TB3	p.Cys982	disulfide bond	folding defect
24	TB3	p.Cys993	disulfide bond	folding defect
24	TB3	p.Cys996	disulfide bond	folding defect
24	TB3	p.Cys1008	disulfide bond	folding defect
37-38	TB4	p.Cys1534	disulfide bond	folding defect
37-38	TB4	p.Cys1549	disulfide bond	folding defect
37-38	TB4	p.Cys1562	disulfide bond	folding defect
37-38	TB4	p.Cys1563	disulfide bond	folding defect
37-38	TB4	p.Cys1564	disulfide bond	folding defect
37-38	TB4	p.Cys1574	disulfide bond	folding defect
37-38	TB4	p.Cys1577	disulfide bond	folding defect
37-38	TB4	p.Cys1589	disulfide bond	folding defect

41-42	TB5	p.Cys1695	disulfide bond	folding defect
41-42	TB5	p.Cys1706	disulfide bond	folding defect
41-42	TB5	p.Cys1719	disulfide bond	folding defect
41-42	TB5	p.Cys1720	disulfide bond	folding defect
41-42	TB5	p.Cys1721	disulfide bond	folding defect
41-42	TB5	p.Cys1733	disulfide bond	folding defect
41-42	TB5	p.Cys1736	disulfide bond	folding defect
41-42	TB5	p.Cys1748	disulfide bond	folding defect
50-51	TB6	p.Cys2061	disulfide bond	folding defect
50-51	TB6	p.Cys2070	disulfide bond	folding defect
50-51	TB6	p.Cys2083	disulfide bond	folding defect
50-51	TB6	p.Cys2084	disulfide bond	folding defect
50-51	TB6	p.Cys2085	disulfide bond	folding defect
50-51	TB6	p.Cys2096	disulfide bond	folding defect
50-51	TB6	p.Cys2099	disulfide bond	folding defect
50-51	TB6	p.Cys2111	disulfide bond	folding defect
57	TB7	p.Cys2339	disulfide bond	folding defect
57	TB7	p.Cys2348	disulfide bond	folding defect
57	TB7	p.Cys2363	disulfide bond	folding defect
57	TB7	p.Cys2364	disulfide bond	folding defect
57	TB7	p.Cys2365	disulfide bond	folding defect
57	TB7	p.Cys2375	disulfide bond	folding defect
57	TB7	p.Cys2378	disulfide bond	folding defect
57	TB7	p.Cys2390	disulfide bond	folding defect
7	cbEGF1	p.Gly260	interdomain packaging	folding defect
8	cbEGF2	p.Gly302	interdomain packaging	folding defect
12	cbEGF3	p.Gly502	interdomain packaging	folding defect
13	cbEGF4	p.Gly544	interdomain packaging	folding defect
13	cbEGF4	p.Gly551	interdomain packaging	folding defect
14	cbEGF5	p.Gly585	interdomain packaging	folding defect
14	cbEGF5	p.Gly592	interdomain packaging	folding defect
15	cbEGF6	p.Gly626	interdomain packaging	folding defect
15	cbEGF6	p.Gly633	interdomain packaging	folding defect
18	cbEGF7	p.Gly737	interdomain packaging	folding defect
19	cbEGF8	p.Gly779	interdomain packaging	folding defect
19	cbEGF8	p.Gly786	interdomain packaging	folding defect
20	cbEGF9	p.Gly819	interdomain packaging	folding defect
20	cbEGF9	p.Gly826	interdomain packaging	folding defect
23	cbEGF10	p.Gly924	interdomain packaging	folding defect
25	cbEGF11	p.Gly1042	interdomain packaging	folding defect
26	cbEGF12	p.Gly1084	interdomain packaging	folding defect
26	cbEGF12	p.Gly1091	interdomain packaging	folding defect
27	cbEGF13	p.Gly1127	interdomain packaging	folding defect
27	cbEGF13	p.Gly1134	interdomain packaging	folding defect
28	cbEGF14	p.Gly1169	interdomain packaging	folding defect
28	cbEGF14	p.Gly1176	interdomain packaging	folding defect
29	cbEGF15	p.Gly1217	interdomain packaging	folding defect
30	cbEGF16	p.Gly1252	interdomain packaging	folding defect

30	cbEGF16	p.Gly1259	interdomain packaging	folding defect
31	cbEGF17	p.Gly1294	interdomain packaging	folding defect
31	cbEGF17	p.Gly1301	interdomain packaging	folding defect
32	cbEGF18	p.Gly1344	interdomain packaging	folding defect
33	cbEGF19	p.Gly1385	interdomain packaging	folding defect
34	cbEGF20	p.Gly1418	interdomain packaging	folding defect
34	cbEGF20	p.Gly1425	interdomain packaging	folding defect
35	cbEGF21	p.Gly1459	interdomain packaging	folding defect
35	cbEGF21	p.Gly1466	interdomain packaging	folding defect
36	cbEGF22	p.Gly1500	interdomain packaging	folding defect
36	cbEGF22	p.Gly1507	interdomain packaging	folding defect
39	cbEGF23	p.Gly1620	interdomain packaging	folding defect
40	cbEGF24	p.Gly1661	interdomain packaging	folding defect
40	cbEGF24	p.Gly1668	interdomain packaging	folding defect
43	cbEGF25	p.Gly1780	interdomain packaging	folding defect
44	cbEGF26	p.Gly1829	interdomain packaging	folding defect
45	cbEGF27	p.Gly1863	interdomain packaging	folding defect
45	cbEGF27	p.Gly1870	interdomain packaging	folding defect
46	cbEGF28	p.Gly1903	interdomain packaging	folding defect
46	cbEGF28	p.Gly1910	interdomain packaging	folding defect
47	cbEGF29	p.Gly1945	interdomain packaging	folding defect
47	cbEGF29	p.Gly1952	interdomain packaging	folding defect
48	cbEGF30	p.Gly1987	interdomain packaging	folding defect
48	cbEGF30	p.Gly1994	interdomain packaging	folding defect
49	cbEGF31	p.Gly2027	interdomain packaging	folding defect
49	cbEGF31	p.Gly2034	interdomain packaging	folding defect
52	cbEGF32	p.Gly2140	interdomain packaging	folding defect
53	cbEGF33	p.Gly2179	interdomain packaging	folding defect
53	cbEGF33	p.Gly2186	interdomain packaging	folding defect
54	cbEGF34	p.Gly2226	interdomain packaging	folding defect
55	cbEGF35	p.Gly2270	interdomain packaging	folding defect
56	cbEGF36	p.Gly2305	interdomain packaging	folding defect
56	cbEGF36	p.Gly2312	interdomain packaging	folding defect
58	cbEGF37	p.Gly2416	interdomain packaging	folding defect
59	cbEGF38	p.Gly2464	interdomain packaging	folding defect
60	cbEGF39	p.Gly2505	interdomain packaging	folding defect
61	cbEGF40	p.Gly2546	interdomain packaging	folding defect
62	cbEGF41	p.Gly2586	interdomain packaging	folding defect
63	cbEGF42	p.Gly2627	interdomain packaging	folding defect
64	cbEGF43	p.Gly2668	calcium binding	folding defect
7	cbEGF1	p.Asp246	calcium binding	folding defect
7	cbEGF1	p.Asp248	calcium binding	folding defect
7	cbEGF1	p.Glu249	calcium binding	folding defect
7	cbEGF1	p.Asn264	calcium binding	folding defect
7	cbEGF1	p.Phe269	calcium binding	folding defect
8	cbEGF2	p.Asp288	calcium binding	folding defect
8	cbEGF2	p.Asp290	calcium binding	folding defect

8	cbEGF2	p.Glu291	calcium binding	folding defect
8	cbEGF2	p.Asn306	calcium binding	folding defect
8	cbEGF2	p.Tyr311	calcium binding	folding defect
12	cbEGF3	p.Asp490	calcium binding	folding defect
12	cbEGF3	p.Asp492	calcium binding	folding defect
12	cbEGF3	p.Glu493	calcium binding	folding defect
12	cbEGF3	p.Asn506	calcium binding	folding defect
12	cbEGF3	p.Tyr511	calcium binding	folding defect
13	cbEGF4	p.Asp530	calcium binding	folding defect
13	cbEGF4	p.Asp531	calcium binding	folding defect
13	cbEGF4	p.Glu532	calcium binding	folding defect
13	cbEGF4	p.Asn548	calcium binding	folding defect
13	cbEGF4	p.Phe553	calcium binding	folding defect
14	cbEGF5	p.Asp572	calcium binding	folding defect
14	cbEFG5	p.Asp574	calcium binding	folding defect
14	cbEGF5	p.Glu575	calcium binding	folding defect
14	cbEFG5	p.Asn589	calcium binding	folding defect
14	cbEGF5	p.Phe574	calcium binding	folding defect
15	cbEGF6	p.Asp613	calcium binding	folding defect
15	cbEGF6	p.Asn615	calcium binding	folding defect
15	cbEGF6	p.Glu616	calcium binding	folding defect
15	cbEGF6	p.Asn630	calcium binding	folding defect
15	cbEGF6	p.Tyr635	calcium binding	folding defect
18	cbEGF7	p.Asp723	calcium binding	folding defect
18	cbEGF7	p.Asn725	calcium binding	folding defect
18	cbEGF7	p.Glu726	calcium binding	folding defect
18	cbEGF7	p.Asn741	calcium binding	folding defect
18	cbEGF7	p.Tyr445	calcium binding	folding defect
19	cbEGF8	p.Asp765	calcium binding	folding defect
19	cbEGF8	p.Asn767	calcium binding	folding defect
19	cbEGF8	p.Glu768	calcium binding	folding defect
19	cbEGF8	p.Asn783	calcium binding	folding defect
19	cbEGF8	p.Phe788	calcium binding	folding defect
20	cbEGF9	p.Asp807	calcium binding	folding defect
20	cbEGF9	p.Asp809	calcium binding	folding defect
20	cbEGF9	p.Glu810	calcium binding	folding defect
20	cbEGF9	p.Asn823	calcium binding	folding defect
20	cbEGF9	p.Phe828	calcium binding	folding defect
23	cbEGF10	p.Asp910	calcium binding	folding defect
23	cbEGF10	p.Asp912	calcium binding	folding defect
23	cbEGF10	p.Glu913	calcium binding	folding defect
23	cbEGF10	p.Asn928	calcium binding	folding defect
23	cbEGF10	p.Phe933	calcium binding	folding defect
25	cbEGF11	p.Asp1028	calcium binding	folding defect
25	cbEGF11	p.Asn1030	calcium binding	folding defect
25	cbEGF11	p.Glu1031	calcium binding	folding defect
25	cbEGF11	p.Asn1046	calcium binding	folding defect
25	cbEGF11	p.Phe1051	calcium binding	folding defect

26	cbEGF12	p.Asp1070	calcium binding	folding defect
26	cbEGF12	p.Asp1072	calcium binding	folding defect
26	cbEGF12	p.Glu1073	calcium binding	folding defect
26	cbEGF12	p.Asn1088	calcium binding	folding defect
26	cbEGF12	p.Phe1093	calcium binding	folding defect
27	cbEGF13	p.Asp1113	calcium binding	folding defect
27	cbEGF13	p.Asp1115	calcium binding	folding defect
27	cbEGF13	p.Glu1116	calcium binding	folding defect
27	cbEGF13	p.Asn1131	calcium binding	folding defect
27	cbEGF13	p.Tyr1136	calcium binding	folding defect
28	cbEGF14	p.Asp1155	calcium binding	folding defect
28	cbEGF14	p.Asn1157	calcium binding	folding defect
28	cbEGF14	p.Glu1158	calcium binding	folding defect
28	cbEGF14	p.Asn1173	calcium binding	folding defect
28	cbEGF14	p.Tyr1178	calcium binding	folding defect
29	cbEGF15	p.Asp1197	calcium binding	folding defect
29	cbEGF15	p.Asp1199	calcium binding	folding defect
29	cbEGF15	p.Glu1200	calcium binding	folding defect
29	cbEGF15	p.Asn1214	calcium binding	folding defect
29	cbEGF15	p.Tyr1219	calcium binding	folding defect
30	cbEGF16	p.Asp1238	calcium binding	folding defect
30	cbEGF16	p.Asp1240	calcium binding	folding defect
30	cbEGF16	p.Glu1241	calcium binding	folding defect
30	cbEGF16	p.Asn1256	calcium binding	folding defect
30	cbEGF16	p.Tyr1261	calcium binding	folding defect
31	cbEGF17	p.Asp1280	calcium binding	folding defect
31	cbEGF17	p.Asn1282	calcium binding	folding defect
31	cbEGF17	p.Glu1283	calcium binding	folding defect
31	cbEGF17	p.Asn1298	calcium binding	folding defect
31	cbEGF17	p.Phe1303	calcium binding	folding defect
32	cbEGF18	p.Asp1322	calcium binding	folding defect
32	cbEGF18	p.Asn1324	calcium binding	folding defect
32	cbEGF18	p.Glu1325	calcium binding	folding defect
32	cbEGF18	p.Asn1341	calcium binding	folding defect
32	cbEGF18	p.Phe1346	calcium binding	folding defect
33	cbEGF19	p.Asp1363	calcium binding	folding defect
33	cbEGF19	p.Asp1365	calcium binding	folding defect
33	cbEGF19	p.Glu1366	calcium binding	folding defect
33	cbEGF19	p.Asn1382	calcium binding	folding defect
33	cbEGF19	p.Tyr1387	calcium binding	folding defect
34	cbEGF20	p.Asp1404	calcium binding	folding defect
34	cbEGF20	p.Asp1406	calcium binding	folding defect
34	cbEGF20	p.Glu1407	calcium binding	folding defect
34	cbEGF20	p.Asn1422	calcium binding	folding defect
34	cbEGF20	p.Tyr1427	calcium binding	folding defect
35	cbEGF21	p.Asp1446	calcium binding	folding defect
35	cbEGF21	p.Asp1448	calcium binding	folding defect
35	cbEGF21	p.Glu1449	calcium binding	folding defect

35	cbEGF21	p.Asn1463	calcium binding	folding defect
35	cbEGF21	p.Phe1468	calcium binding	folding defect
36	cbEGF22	p.Asp1487	calcium binding	folding defect
36	cbEGF22	p.Asn1489	calcium binding	folding defect
36	cbEGF22	p.Glu1490	calcium binding	folding defect
36	cbEGF22	p.Asn1504	calcium binding	folding defect
36	cbEGF22	p.Tyr1509	calcium binding	folding defect
39	cbEGF23	p.Asp1606	calcium binding	folding defect
39	cbEGF23	p.Asp1608	calcium binding	folding defect
39	cbEGF23	p.Glu1609	calcium binding	folding defect
39	cbEGF23	p.Asn 1624	calcium binding	folding defect
39	cbEGF23	p.Phe1629	calcium binding	folding defect
40	cbEGF24	p.Asp1648	calcium binding	folding defect
40	cbEGF24	p.Asn1650	calcium binding	folding defect
40	cbEGF24	p.Glu1651	calcium binding	folding defect
40	cbEGF24	p.Asn1665	calcium binding	folding defect
40	cbEGF24	p.Tyr1670	calcium binding	folding defect
43	cbEGF25	p.Asp1766	calcium binding	folding defect
43	cbEGF25	p.Asp1768	calcium binding	folding defect
43	cbEGF25	p.Glu1769	calcium binding	folding defect
43	cbEGF25	p.Asn1784	calcium binding	folding defect
43	cbEGF25	p.Phe1789	calcium binding	folding defect
44	cbEGF26	p.Asp1808	calcium binding	folding defect
44	cbEGF26	p.Asp1810	calcium binding	folding defect
44	cbEGF26	p.Glu1811	calcium binding	folding defect
44	cbEGF26	p.Asn1826	calcium binding	folding defect
44	cbEGF26	p.Tyr1831	calcium binding	folding defect
45	cbEGF27	p.Asp1849	calcium binding	folding defect
45	cbEGF27	p.Asn1851	calcium binding	folding defect
45	cbEGF27	p.Glu1852	calcium binding	folding defect
45	cbEGF27	p.Asp1867	calcium binding	folding defect
45	cbEGF27	p.Phe1872	calcium binding	folding defect
46	cbEGF28	p.Asp1891	calcium binding	folding defect
46	cbEGF28	p.Asn1893	calcium binding	folding defect
46	cbEGF28	p.Glu1894	calcium binding	folding defect
46	cbEGF28	p.Asn1907	calcium binding	folding defect
46	cbEGF28	p.Phe1912	calcium binding	folding defect
47	cbEGF29	p.Asp1930	calcium binding	folding defect
47	cbEGF29	p.Asp1932	calcium binding	folding defect
47	cbEGF29	p.Glu1933	calcium binding	folding defect
47	cbEGF29	p.Asn1949	calcium binding	folding defect
47	cbEGF29	p.Phe1954	calcium binding	folding defect
48	cbEGF30	p.Asp1973	calcium binding	folding defect
48	cbEGF30	p.Asn1975	calcium binding	folding defect
48	cbEGF30	p.Glu1976	calcium binding	folding defect
48	cbEGF30	p.Asn1991	calcium binding	folding defect
48	cbEGF30	p.Tyr1996	calcium binding	folding defect
49	cbEGF31	p.Asp2013	calcium binding	folding defect

49	cbEGF31	p.Asp2015	calcium binding	folding defect
49	cbEGF31	p.Glu2016	calcium binding	folding defect
49	cbEGF31	p.Asn2031	calcium binding	folding defect
49	cbEGF31	p.Phe2036	calcium binding	folding defect
52	cbEGF32	p.Asp2127	calcium binding	folding defect
52	cbEGF32	p.Asp2129	calcium binding	folding defect
52	cbEGF32	p.Glu2130	calcium binding	folding defect
52	cbEGF32	p.Asn2144	calcium binding	folding defect
52	cbEGF32	p.Tyr2149	calcium binding	folding defect
53	cbEGF33	p.Asp2166	calcium binding	folding defect
53	cbEGF33	p.Asp2168	calcium binding	folding defect
53	cbEGF33	p.Glu2169	calcium binding	folding defect
53	cbEGF33	p.Asn2183	calcium binding	folding defect
53	cbEGF33	p.Phe2188	calcium binding	folding defect
54	cbEGF34	p.Asp2206	calcium binding	folding defect
54	cbEGF34	p.Asn2208	calcium binding	folding defect
54	cbEGF34	p.Glu2209	calcium binding	folding defect
54	cbEGF34	p.Asn2223	calcium binding	folding defect
54	cbEGF34	p.Tyr2228	calcium binding	folding defect
55	cbEGF35	p.Asp2247	calcium binding	folding defect
55	cbEGF35	p.Asp2249	calcium binding	folding defect
55	cbEGF35	p.Glu2250	calcium binding	folding defect
55	cbEGF35	p.Asn2267	calcium binding	folding defect
55	cbEGF35	p.Tyr2272	calcium binding	folding defect
56	cbEGF36	p.Asp2291	calcium binding	folding defect
56	cbEGF36	p.Asn2293	calcium binding	folding defect
56	cbEGF36	p.Glu2294	calcium binding	folding defect
56	cbEGF36	p.Asn2309	calcium binding	folding defect
56	cbEGF36	p.Tyr2314	calcium binding	folding defect
58	cbEGF37	p.Asp2402	calcium binding	folding defect
58	cbEGF37	p.Asp2404	calcium binding	folding defect
58	cbEGF37	p.Glu2405	calcium binding	folding defect
58	cbEGF37	p.Asn2420	calcium binding	folding defect
58	cbEGF37	p.Tyr2425	calcium binding	folding defect
59	cbEGF38	p.Asp2444	calcium binding	folding defect
59	cbEGF38	p.Asn2446	calcium binding	folding defect
59	cbEGF38	p.Glu2447	calcium binding	folding defect
59	cbEGF38	p.Asn2461	calcium binding	folding defect
59	cbEGF38	p.Tyr2466	calcium binding	folding defect
60	cbEGF39	p.Asp2485	calcium binding	folding defect
60	cbEGF39	p.Asp2487	calcium binding	folding defect
60	cbEGF39	p.Glu2488	calcium binding	folding defect
60	cbEGF39	p.Asn2502	calcium binding	folding defect
60	cbEGF39	p.Phe2507	calcium binding	folding defect
61	cbEGF40	p.Asp2524	calcium binding	folding defect
61	cbEGF40	p.Asn2526	calcium binding	folding defect
61	cbEGF40	p.Glu2527	calcium binding	folding defect
61	cbEGF40	p.Asn2543	calcium binding	folding defect

61	cbEGF40	p.Phe2548	calcium binding	folding defect
62	cbEGF41	p.Asp2567	calcium binding	folding defect
62	cbEGF41	p.Asp2569	calcium binding	folding defect
62	cbEGF41	p.Glu2570	calcium binding	folding defect
62	cbEGF41	p.Asn2583	calcium binding	folding defect
62	cbEGF41	p.Tyr2588	calcium binding	folding defect
63	cbEGF42	p.Asp2607	calcium binding	folding defect
63	cbEGF42	p.Asn2609	calcium binding	folding defect
63	cbEGF42	p.Glu2610	calcium binding	folding defect
63	cbEGF42	p.Asn2624	calcium binding	folding defect
63	cbEGF42	p.Tyr2629	calcium binding	folding defect
63-64	cbEGF43	p.Asp2648	calcium binding	folding defect
63-64	cbEGF43	p.Asn2650	calcium binding	folding defect
63-64	cbEGF43	p.Glu2651	calcium binding	folding defect
63-64	cbEGF43	p.Asn2665	calcium binding	folding defect
63-64	cbEGF43	p.Tyr2670	calcium binding	folding defect